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Original Article

CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC ANALYSIS OF BIOACTIVE COMPOUNDS FROM *CAYRATIA TRIFOLIA* (L.) STEM

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ABSTRACT

Objective: The present study was to analyze the bioactive compounds from stem ethanolic extract of *Cayratia trifolia* by FTIR, HPTLC and GC-MS techniques.

Methods: The FTIR was applied and infrared spectrum in mid-infrared region 4000-400 $^{cm-1}$ was used, HPTLC fingerprinting profiles was done by using Hamilton syringe and CAMAG LINOMAT 5 instrument and GC-MS analysis of stem ethanolic extract of *Cayratia trifolia* (L.) was performed using the equipment Agilent technologies 7890 A.

Results: The FTIR analysis identified the functional groups such as amine, acid, alkane, ketone acyclic, carbonyl, aromatic, ester and alkene. HPTLC fingerprinting profile proves the presence of alkaloids, flavonoids, glycosides, saponin and steroids. GC-MS revealed the presence of various compounds like hexadecanoic acid-ethylester, phytol, tetratetracontane, stigmasterol, nonacosane and octadecane-1-bromo-in stem ethanolic extract of *Cayratia trifolia*.

Conclusion: In conclusion, *Cayratia trifolia* plant stems ethanolic extract holds more bioactive compounds that may lead to the development of novel drug against various diseases and disorders.

Keywords: *Cayrtaia trifolia*, FTIR Spectroscopy, HPTLC analysis, GC-MS technique, Bioactive compound

INTRODUCTION

Medicinal plants are very ancient and true natural medicines which are useful for the treatment of different diseases. They can be used directly or in extracted forms for the management of various ailments due to the presence of various secondary metabolites [1]. The use of plants in the traditional remedy of many other cultures has been widely documented. These plant-based systems continue to play a significant role in health care and it has been projected by the World Health Organization that around 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care, although plant products also play a main role in the health care systems of the remaining 20% of the population mostly residing in developed countries [2]. Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leading to for the treatment and prevention of diseases [3, 4].

The valuable medicinal properties of different plants are due to the presence of several antioxidants like saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones and terpenoids [5]. Currently, the synthetic antioxidants might be unsafe and its toxicity has been criticized. It is generally assumed that frequent use of plant-derived phytochemicals may contribute to shift the stability in the direction of a sufficient antioxidant status. As a result, attention in natural antioxidants, in particular, plant origin, has deeply amplified in recent years [6].

Cayratia trifolia (L.) is a medicinal plant which belongs to the family of Vitaceae; It has been reported to contain a huge amount of bioactive compounds such as yellow waxy oil, steroids, terpenoids, flavonoids and tannins [7, 8]. Stem, leaves, and roots are reported to possess hydrocyanic acid and delphinidin. Several flavonoids such as cyanidins are reported in the leaves [9]. Infusion of seeds along with extract of tubers is traditionally given orally to diabetic patients to check sugar level of blood. The whole plant is used in diuretics, tumors, neuralgia and splenopathy [10]. The paste of tubers is applied on the affected part in the treatment of snake bite. It is reported to possess antiviral, antibacterial, antiprotozoal, hypoglycaemic, anticancer and diuretic activity, etc [11]. The ethanolic extract of *Cayratia trifolia* possesses a good free radical scavenging activity which may be due to the presence of alkaloids and flavonoids [12]. The aim of this study is to establish the bioactive compounds present in the *Cayratia trifolia* stem ethanolic extract with the aid of FTIR, HPTLC, and GC MS techniques.

MATERIALS AND METHODS

Collection of plant material

The stem parts of *Cayratia trifolia* (L.) was collected from in and around the area of Kumbakonam, Tamil Nadu, India and it was authenticated by Dr. P. Sathyanarayanan, Botanical survey of India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/ 5/23/2010-2011/Tech.1527 [12]. The fresh stem parts of plant material was washed under the running tap water, dipped on saline overnight, air dried and finely powdered for further use.

Extraction preparation

100g of dried plant powder was extracted in 500 ml of ethanol in a sporadic shaker for 72 h at room temperature. The extract was collected and concentrated at 40 ° C under reduced pressure using rotary evaporator. The dried extract was stored at 4 ° C until further compound isolation process.

Instrumentation

FTIR spectroscopy analysis

The ethanol extract of *Cayratia trifolia* (L.) was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded on a Shimadzu FTIR Spectrometer in the region of 4,000-400 cm^{-1}. The detector used in the system was standard DLATGS with a mirror speed of 2.8 mm/sec [13].

HPTLC analysis

2 µl of the sample and 2 µl of standard solution were loaded as 5 mm band length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton

syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapour) with respective mobile phases (Alkaloid, Flavonoid, Glycoside, Steroid and saponin) and the plate was developed in the respective mobile phase up to 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV366 nm. The developed plate was sprayed with respective spray reagent and dried at 100 °C in Hot air oven. The plate was photo-documented at Daylight and UV 366 nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500 nm. The Peak table, Peak display and Peak densitogram were noted.

Table 1: Mobile phases and spray reagents of HPTLC profiles

Profile name	Mobile phases	Spray reagent
Alkaloid	Ethyl acetate-methanol-water (10: 1.35: 1)	Dragendorff's reagent followed by 10% Ethanolic sulphuric acid reagent
Flavonoid	Ethyl acetate-Butanone-Formic acid-Water (5:3:1:1)	1% Ethanolic Aluminium chloride reagent
Glycosides	Ethyl acetate–Ethanol-Water (8:2:1:2)	Liberman-Burchard reagent
Saponin	Chloroform–Glacial acetic acid–Methanol–Water (6.4:3.2:1.2:0.8)	Anisaldehyde sulphuric acid reagent
Steroids	Toluene-Acetone (9:1)	Anisaldehyde sulphuric acid reagent.

GC-MS analysis

GC-MS analysis of stem ethanolic extract of *Cayratia trifolia* (L.) was performed using the equipment Agilent technologies 7890 A. The equipment has a DB 35–MS Capillary Standard non-polar column with dimensions of 30 mm×0.25 mm ID×0.25µm film. Helium was used as a carrier gas with a flow rate of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was based on Willey and NIST libraries as well as a comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results obtained have been tabulated [14].

RESULTS AND DISCUSSION

Plants are the important source of functional components for the development of new chemotherapeutic agents [15]. The pharmacological activities of any plant samples are due to the presence of secondary metabolites. [5]. Studies have shown that many antioxidant compounds possess anti-inflammatory, antitumor, anticarcinogenic, antibacterial, and antiviral activities [16, 17]. Natural and some synthetic compounds can prevent, suppress, or reverse the progression of cancer [18]. The bark extract of *Cayratia trifolia* (L.) has been reported to have antiviral and anticancer activities in animal models [19]. Therefore, this study was focused on analyzing bioactive compounds against various cancers.

FT-IR spectrum reflecting objectively the panorama of chemical constituents in a complex system is the most probable method to validate and identify the substance systems such as traditional medicine and herbal medicine [20]. This spectrum was useful for the compound identification and when to run under IR region in the range of 400-4000 $^{cm-1}$there was a variation in the peaks in plant samples [21]. The stem extract of *Cayratia trifolia* was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. Fig. 1 and table 2 show the strong peaks at 3371 cm^{-1}corresponds to amine N-H stretching, 2926 cm^{-1}exists acid O-H stretching group, 2856 cm^{-1} belongs to alkane C-H stretching vibration that are mainly generated by lipids [22], 1714 cm^{-1} and 1647 cm^{-1} corresponds to C=O stretching indicate the presence of acid, carbonyl and ketone acyclic groups in *Cayratia trifolia*. The more intense bands occurring at 1456, 1317, 1074 and 835 corresponds to C=C aromatic,-C-H alkane, C-O ester and =C-H alkene stretching groups in *Cayratia trifolia*. Based on the functional group analysis, *Cayratia trifolia* stem part doesn't contain any toxic compounds.

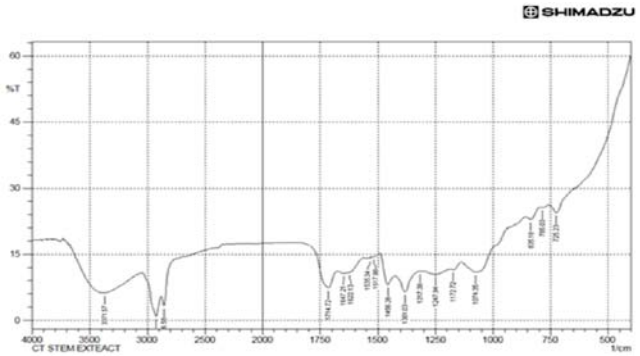

Fig. 1: FTIR Spectrum analysis of stem ethanolic extract of *Cayratia trifolia*

Table 2: FTIR spectrum peak values and functional groups of stem ethanolic extract of *Cayratia trifolia*

Functional groups		Type of vibration	Characteristic absorptions (cm^{-1})
N-H	Amine	Stretch	3371
O-H	Acid	Stretch	2926
C-H	Alkane	Stretch	2856
C=O	Acid/Ketone acyclic	Stretch	1714
C=O	Carbonyl	Stretch	1647
C=C	Aromatic	Stretch	1456
-C-H	Alkane	Stretch	1317
C-O	Ester	Stretch	1074
=C-H	Alkene	Stretch	835

Fingerprint analysis by HPTLC has become a valuable and powerful tool for linking the chemical constituent profile of the plants with botanical identity and for the estimation of chemical and biochemical markers [23]. HPTLC analysis has increasing interest in discovery of natural antioxidants, especially those of plant origin. In traditional medicines, medicinal plants have contributed hugely to the traditional and western medicines through providing ingredients for drugs or having played central roles in the drug discovery [24]. Phytoconstituents such as alkaloids, flavanoids, tannins, phenols, saponins, steroids, terpenoids and several other

aromatic compounds in the plants serve as defense mechanism against various diseases [25]. Alkaloids are known to be effective for antihypertensive properties [26]. Table 3 shows the presence of various alkaloids and unknown compounds with its Rf values. Yellow, Brownish-yellow coloured zone at visible mode was present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of alkaloid or nitrogen containing compound in the given standard and may be in the sample. Colchicine was used as standard and it produced a clear zone with Rf value 0.53. *Cayratia trifolia* plant shows the presence of two alkaloid compounds and twelve unknown compounds with Rf values 0.65, 0.76 and 0.04, 0.06, 0.09, 0.12, 0.18, 0.24, 0.33, 0.36, 0.41, 0.43, 0.48 respectively. Chromatogram and densitogram were observed under daylight as well as in ultra violet mode which is represented in fig. 2 and fig. 3.

Table 3: Retention factor (Rf), height and area of peaks of Alkaloids

Track	Peak	Rf	Height	Area	Assigned substance
Sample C. T	1	0.04	15.4	158.7	Unknown
Sample C. T	2	0.06	229.2	1978.6	Unknown
Sample C. T	3	0.09	34.7	674.1	Unknown
Sample C. T	4	0.12	51.2	820.5	Unknown
Sample C. T	5	0.18	31.7	181.0	Unknown
Sample C. T	6	0.24	13.9	239.9	Unknown
Sample C. T	7	0.33	45.1	1331.4	Unknown
Sample C. T	8	0.36	35.3	789.5	Unknown
Sample C. T	9	0.41	33.7	679.0	Unknown
Sample C. T	10	0.43	42.6	738.0	Unknown
Sample C. T	11	0.48	65.0	933.2	Unknown
Sample C. T	12	0.53	15.9	439.4	Unknown
Sample C. T	13	0.65	56.3	2018.3	Alkaloid/Nitrogen containing compound 1
Sample C. T	14	0.76	110.1	2881.6	Alkaloid/Nitrogen containing compound 2
STD	1	0.53	101.0	2217.3	Colchicine

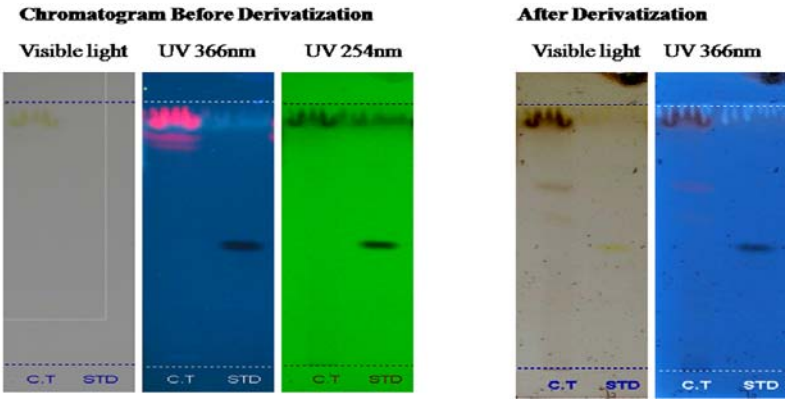

Fig. 2: HPTLC Chromatogram of Alkaloids in stem ethanolic extract of *Cayratia trifolia*

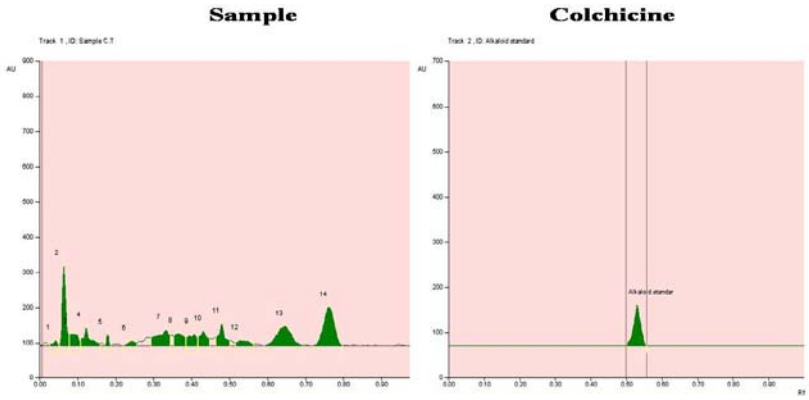

Fig. 3: Densitogram display for alkaloids in stem ethanolic extract of *Cayratia trifolia*

Many flavonoids and related compounds are reported to possess strong antioxidative characteristics [27]. Numerous preclinical and some clinical studies suggest that flavonoids have the potential for the prevention and treatment of several diseases. Some epidemiological studies support a protective role of diets rich in foods with flavonoids and a reduced risk of developing cancer [28]. Table 4, fig. 4 and 5 shows the flavonoid profile of stem ethanolic extract of *Cayratia trifolia* compared with standard rutin along with peak chromatogram and densitogram. Yellow or Yellowish-blue coloured fluorescent zone at UV 366 nm mode

were present in the tracks; it was observed from the chromatogram after derivatization, which confirmed the presence of Flavonoid. The standard produced a clear zone with Rf value 0.51. Five unknown compounds and two flavonoid compounds were present in our plant sample with Rf values 0.07, 0.12, 0.14, 0.51 and 0.29, 0.39 respectively.

Table 4: Retention factor (Rf), height and area of peaks of Flavonoids

Track	Peak	Rf	Height	Area	Assigned substance
Sample C. T	1	0.07	80.4	1223.7	Unknown
Sample C. T	2	0.12	23.5	496.8	Unknown
Sample C. T	3	0.14	21.1	783.7	Unknown
Sample C. T	4	0.29	32.2	1265.3	Flavonoid 1
Sample C. T	5	0.39	14.7	444.3	Flavonoid 2
Sample C. T	6	0.51	12.0	233.3	Unknown
Sample C. T	7	0.73	14.3	183.2	Unknown
STD	1	0.51	464.3	20365.2	Rutin

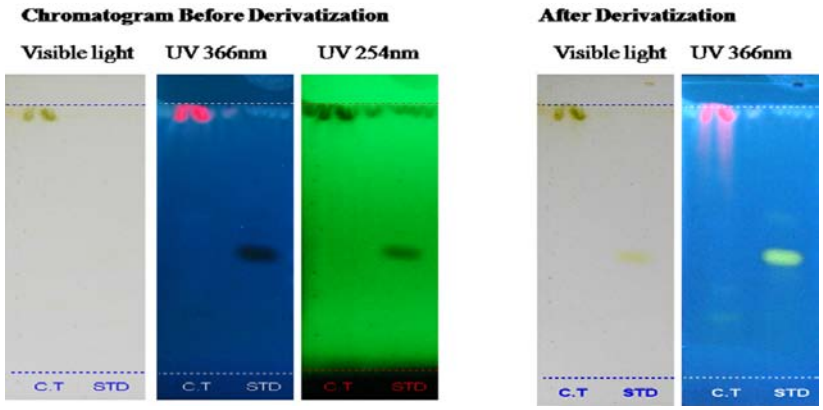

Fig. 4: HPTLC Chromatogram of Flavonoids in stem ethanolic extract of *Cayratia trifolia*

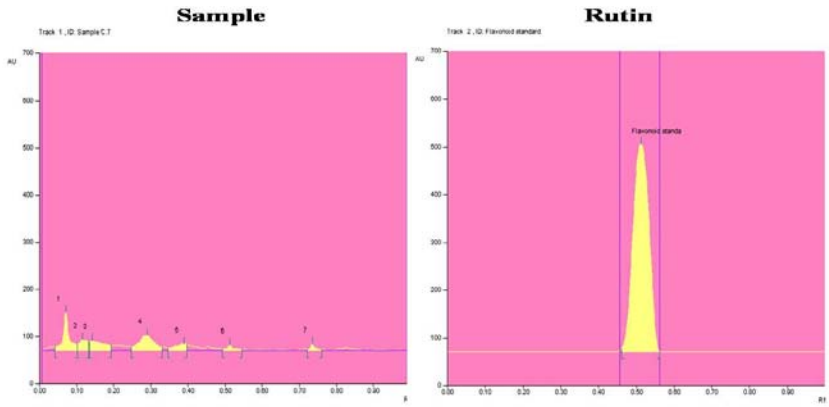

Fig. 5: Densitogram display for Flavonoids in stem ethanolic extract of *Cayratia trifolia*

Table 5: Retention factor (Rf), height and area of peaks of glycosides

Track	Peak	Rf	Height	Area	Assigned substance
Sample C. T	1	0.02	18.3	201.4	Unknown
Sample C. T	2	0.07	178.9	7054.0	Unknown
Sample C. T	3	0.27	43.1	1734.7	Glycoside 1
Sample C. T	4	0.39	20.4	553.6	Unknown
Sample C. T	5	0.45	17.0	611.4	Unknown
Sample C. T	6	0.69	11.6	298.8	Unknown
Sample C. T	7	0.85	22.4	622.6	Unknown
Sample C. T	8	0.94	130.3	2463.3	Glycoside 2
STD	1	0.76	252.6	8363.1	Swertiamarin

Alkaloids, flavonoids, and glycosides have been reported to expert several biological effects like anti-inflammatory, antiallergic, antioxidant, antidiabetic, anti-viral and anticancer activities [29]. *Cayratia trifolia* showed significant antioxidant potential due to the presence flavonoids, tannins, phenols, amino acids, proteins, terpenoids, glycosides, saponin and steroids

[30]. Glycoside compounds are containing a carbohydrate and non-carbohydrates residue in the same molecule. They are important in medicine because of their action on the heart and are used in cardiac insufficiency [31]. Table 5 and fig. 6 and 7 show the presence of various glycosides and unknown compounds with Rf values. The standard swertiamarin produced a clear zone with Rf value 0.76. *Cayratia trifolia* shows the presence of two glycoside compounds and six unknown compounds with R_f values 0.27, 0.94 and 0.02, 0.07, 0.39, 0.45, 0.69, 0.85 respectively.

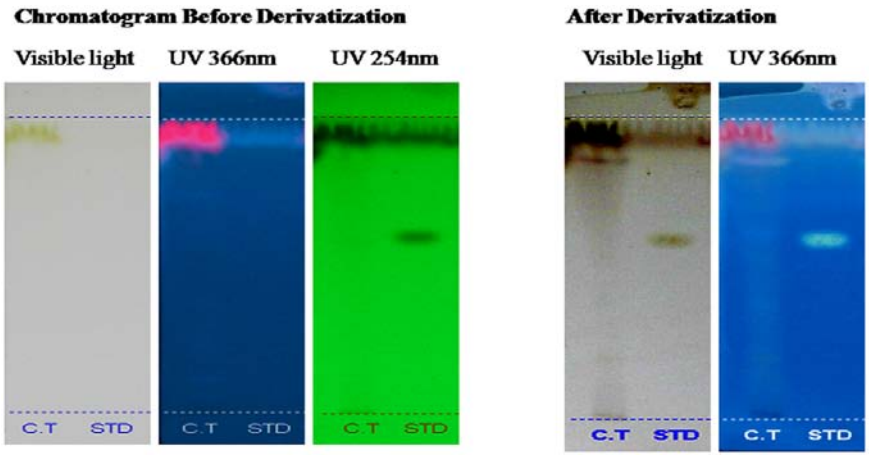

Fig. 6: HPTLC chromatogram of glycosides in stem ethanolic extract of *Cayratia trifolia*

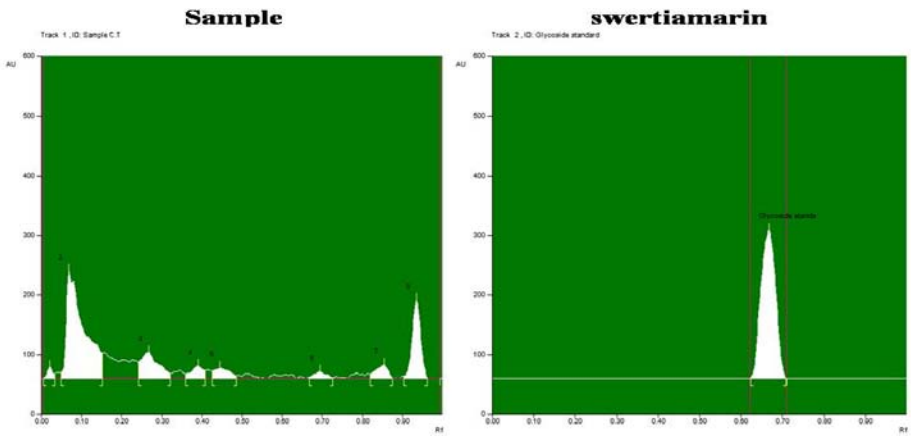

Fig. 7: Densitogram display for glycosides in stem ethanolic extract of *Cayratia trifolia*

Saponins are generally identified as non-volatile, surface active compounds that are widely distributed in nature, occurring primarily in the plant kingdom [32]. Saponins have a various range of properties, which include sweetness and bitterness [33], foaming and emulsifying properties [34], pharmacological and medicinal properties [35], strong haemolytic properties, as well as antimicrobial and cytotoxic activities [36].

Table 6 and fig. 7 and 8 shows the saponin profile of stem ethanolic extract of *Cayratia trifolia* compared with standard saponin along with peak chromatogram and densitogram. *Cayratia trifolia* showed two saponin seven unknown compounds with the Rf values of 0.47, 0.88 and 0.01, 0.07, 0.10, 0.36, 0.66, 0.73, 0.96. Three saponin standards were used, and the Rf values are 0.25, 0.27 and 0.44.

Table 6: Retention factor (Rf), height and area of peaks of saponins

Track	Peak	Rf	Height	Area	Assigned substance
Sample C. T	1	0.01	72.4	570.6	Unknown
Sample C. T	2	0.07	179.7	2556.6	Unknown
Sample C. T	3	0.10	59.3	1668.9	Unknown
Sample C. T	4	0.36	23.7	153.9	Unknown
Sample C. T	5	0.47	22.0	848.1	Saponin 1
Sample C. T	6	0.66	10.8	137.7	Unknown
Sample C. T	7	0.73	13.0	475.7	Unknown
Sample C. T	8	0.88	208.4	6691.1	Saponin 2
Sample C. T	9	0.96	168.1	4346.7	Unknown

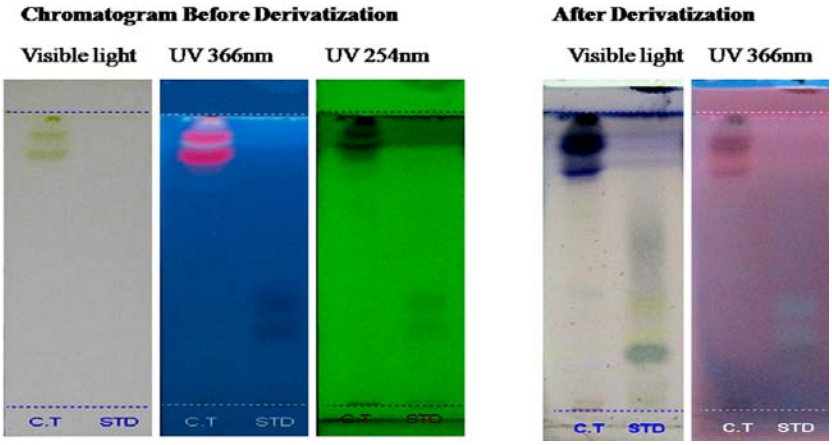

Fig. 8: HPTLC chromatogram of saponins in stem ethanolic extract of *Cayratia trifolia*

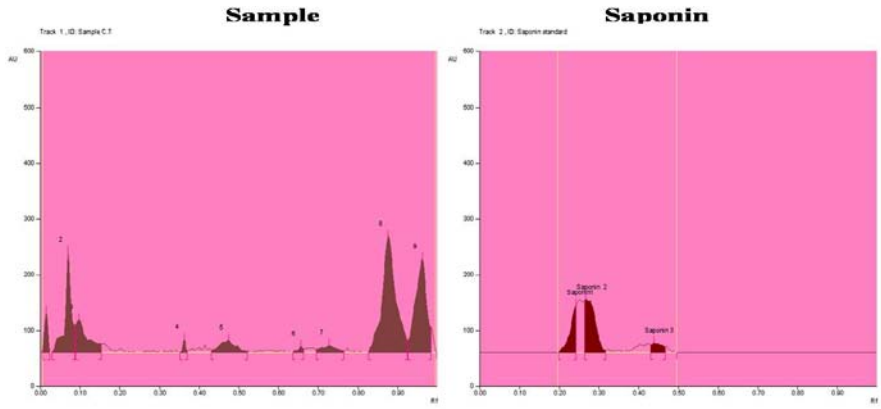

Fig. 9: Densitogram display for saponins in stem ethanolic extract of *Cayratia trifolia*

Steroids have been reported that they have antibacterial properties and association between membrane lipids and sensitivity for steroidal compound which correlates with membrane lipid and in turn exerts action by causing leakages from liposomes [37]. Steroidal compounds are importance and interest in pharmacy due to their relationship through such compounds as sex hormones and promote immune function in the skin and also reduce inflammation [38]. Table 7 and fig. 10 and 11 shows the steroids profile of stem ethanolic extract of *Cayratia trifolia* compared with standard stigmasterol along with peak chromatogram and densitogram. Blue, bluish violet coloured zones at Visible light mode present in the given standard and samples track observed in the chromatogram after derivatization, confirmed the presence of steroid.

The standard produced a clear zone with Rf value 0.47. Seven unknown compounds and three steroid compounds were present in our plant sample with Rf values 0.02, 0.07, 0.08, 0.36, 0.56, 0.81, 0.88 and 0.38, 0.49, 0.72 respectively.

Table 7: Retention factor (Rf), height and area of peaks of Steroids

Track	Peak	Rf	Height	Area	Assigned substance
Sample C. T	1	0.02	166.1	1483.2	Unknown
Sample C. T	2	0.07	274.9	2415.7	Unknown
Sample C. T	3	0.08	178.5	2280.3	Unknown
Sample C. T	4	0.36	207.1	11917.7	Unknown
Sample C. T	5	0.38	207.8	7075.9	Steroid 1
Sample C. T	6	0.49	251.5	12200.3	Steroid 2
Sample C. T	7	0.56	207.0	7285.7	Unknown
Sample C. T	8	0.72	19.0	542.4	Steroid 3
Sample C. T	9	0.81	17.3	381.6	Unknown

The analysis and extraction of plant material play a central role in the development, modernization and quality control of herbal formulations. Study of medicinal plants also facilitates to comprehend plant toxicity and also helps to protect human and animals from natural poisons [14]. GC-MS technique is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters, etc. The GC-MS analysis of *Cayratia trifolia* stems ethanolic extract revealed the presence of twenty bioactive compounds that could contribute to the medicinal quality of the plant. The identification of the bioactive compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in table 8 and fig. 12.

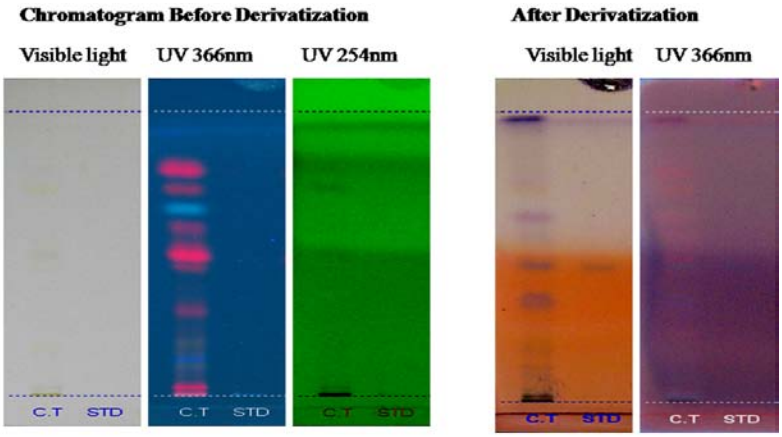

Fig. 10: HPTLC Chromatogram of steroids in stem ethanolic extract of *Cayratia trifolia*

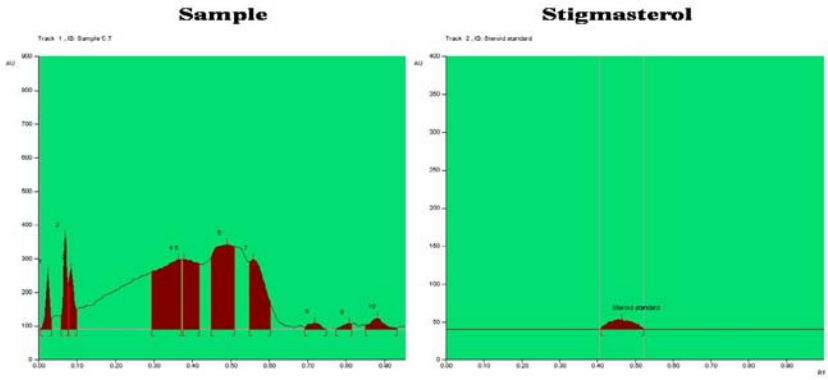

Fig. 11: Densitogram display for Steroids in stem ethanolic extract of *Cayratia trifolia*

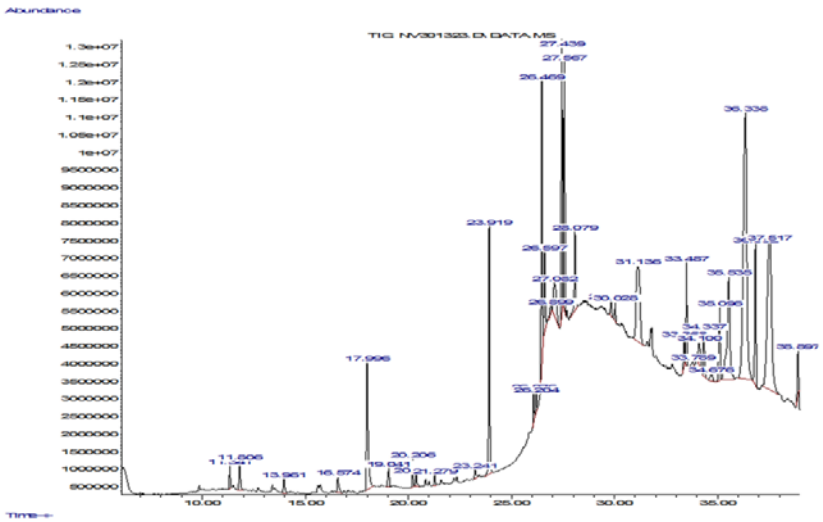

Fig. 12: GC-MS chromatogram graph of stem ethanolic extract of *Cayratia trifolia*

Among the identified bioactive compounds hexadecanoic acid ethyl ester, phytol, tetratriacontane, stigmasterol, nonacosane and octadecane, the 1-bromo-were present high percentage in *Cayratia trifolia*. The structural and kinetics studies of hexadecanoic acid reveal that it is an inhibitor of phospholipase A and confirmed as an anti-inflammatory compound [39]. Phytol is suggested to be a diterpene compound, and it may act as an antimicrobial, anti-inflammatory, anticancer, diuretic [40-42]. Stigmasterol is used as a precursor in the manufacture of semisynthetic progesterone [43] a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D3 [44]. Research has indicated that stigmasterol may be useful in the prevention of certain cancers, including ovarian, prostate, breast, and colon cancers [45]. Octadecane is recommended as ether and acts as an antisepsis [46]. Due to the presence of bioactive compounds in the stem ethanolic extract of *Cayratia trifolia*, it may be used in various pharmaceutical and industrial applications.

Table 8: GC-MS analysis peaks of stem parts of ethanolic extract of *Cayratia trifolia*

Peak	RT (min)	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area %
1.	11.806	Phenyl, 2,4-bis(1,1-dimethylethyl)	$C_{42}H_{63}O_3$	633	0.86
2.	17.996	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-,(R)-	$C_{15}H_{22}O$	218.33	4.67
3.	19.041	Methyl 2,6-dimethyltridecanoate	$C_{16}H_{32}O_2$	256.42	0.58
4.	20.206	Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl-, (1. alpha.,2. beta.,5. alpha.)	$C_{10}H_{18}$	138.24	0.61
5.	23.919	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.47	6.32
6.	26.076	9,12-octadecadienoic, methyl ester	$C_{19}H_{34}O_2$	294.47	0.64
7.	26.469	Phytol	$C_{20}H_{40}O$	296.53	6.51
8.	27.439	Linoleic acid ethylester	$C_{20}H_{34}O_2$	306.5	5.65
9.	27.567	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.44	5.34
10.	28.079	Eicosanoic acid	$C_{20}H_{40}O_2$	312.53	2.29
11.	31.136	Nonacosane	$C_{29}H_{60}$	408.6	6.28
12.	33.368	Z-11-Tetradecen-1-ol trifluoroacetate	$C_{16}H_{27}F_3O_2$	308.37	0.62
13.	34.100	Z,E-3,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266.46	1.02
14.	34.337	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[diethylamino)-ethoxy]fluorene	$C_{30}H_{42}Cl_2N_4O_3$	640	1.05
15.	35.096	Heptacosane, 1-chloro-	$C_{27}H_{55}Cl$	415.17	1.49
16.	35.535	Octadecane, 1-bromo-	$C_{18}H_{37}Br$	333.39	6.38
17.	36.338	Tetratetracontane	$C_{44}H_{90}$	619.18	20.04
18.	36.832	Heptadecane	$C_{17}H_{36}$	240.46	3.61
19.	37.517	Stigmasterol	$C_{29}H_{48}O$	412.69	14.19
20.	38.897	Heptadecane	$C_{17}H_{36}$	240.46	1.09

CONCLUSION

Based on the present study concluded that the *Cayratia trifolia (L.)* plant stems ethanolic extract holds more phytochemicals and bioactive compounds which were confirmed by using FTIR, HPTLC and GC-MS analysis. In future, these bioactive compounds will be helpful in the identification and quality control of the drug and ensure therapeutic need.

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CONFLICT OF INTERESTS

We declare that; we have no conflict of interest

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